

PF 54200

53

We claim:

1. The use of malate dehydrogenase in a method for identifying herbicides, wherein the malate dehydrogenase is a glyoxysomal malate dehydrogenase which is encoded by a nucleic acid sequence comprising:
 - a) a nucleic acid sequence with the nucleic acid sequence shown in SEQ ID NO:1, or
 - b) a nucleic acid sequence with the nucleic acid sequence shown in SEQ ID NO:2, or
 - c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:3 by backtranslating, or
 - d) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by backtranslating the amino acid sequence of a functional equivalent of SEQ ID NO:3, which has at least 66% identity with SEQ ID NO:3.
2. A plant nucleic acid sequence encoding a glyoxysomal malate dehydrogenase comprising:
 - a) a nucleic acid sequence with the nucleic acid sequence shown in SEQ ID NO:1, or
 - b) a nucleic acid sequence with the nucleic acid sequence shown in SEQ ID NO:2, or
 - c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:3 by backtranslating, or
 - d) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by backtranslating the amino acid sequence of a functional equivalent of SEQ ID NO:3, which has at least 79% identity with SEQ ID NO:3.
 - e) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by

AMENDED SHEET

backtranslating the amino acid sequence of a functional equivalent of SEQ ID NO:3, which has at least 87% identity with SEQ ID NO:3.

- 5 3. A polypeptide with the biological activity of a glyoxysomal malate dehydrogenase as target for herbicides, encoded by a nucleic acid molecule according to claim 2.
- 10 4. A method for detecting functional analogs of SEQ ID NO:2
 - a) by generating a probe followed by subsequently screening a genomic library or cDNA library of the species in question, or
 - 15 b) by means of a computer search for analogous sequences in electronic databases.
- 20 5. An expression cassette comprising
 - a) genetic control sequences in operable linkage with a nucleic acid sequence according to claim 2, or
 - 25 b) additional functional elements, or
 - 30 c) a combination of a) and b).
- 35 6. A vector comprising an expression cassette according to claim 5.
- 40 7. A transgenic organism comprising at least one nucleic acid sequence encoding a polypeptide with the biological activity of a glyoxysomal malate dehydrogenase according to claim 2, an expression cassette according to claim 5 or a vector according to claim 6, selected from among bacteria, yeasts, fungi, animal cells or plant cells.
- 45 8. A method for identifying substances with herbicidal activity, comprising the following steps:
 - i. bringing malate dehydrogenase into contact with one or more test compounds under conditions which permit the test compound(s) to bind to the nucleic acid molecule or to the glyoxysomal malate dehydrogenase, and

- ii. detecting whether the test compound binds to the malate dehydrogenase of i), or
- 5 iii. detecting whether the test compound reduces or blocks the enzymatic or biological activity of the malate dehydrogenase of i), or
- 10 iv. detecting whether the test compound reduces or blocks the transcription, translation or expression of the malate dehydrogenase of i),
- the malate dehydrogenase being encoded by a nucleic acid sequence which comprises
- 15 a) a nucleic acid sequence according to claim 2, or
- b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by backtranslating the amino acid sequence of a
- 20 functional equivalent of SEQ ID NO:3, which has at least 63% identity with SEQ ID NO:3.
9. A method according to claim 8, wherein the malate dehydrogenase is a glyoxysomal malate dehydrogenase and is encoded by a nucleic acid sequence which comprises
- 25 a) a nucleic acid sequence according to claim 2, or
- b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by backtranslating the amino acid sequence of a
- 30 functional equivalent of SEQ ID NO:3, which has at least 66% identity with SEQ ID NO:3.
- 35 10. A method according to claim 8 or 9, wherein a test compound is selected which reduces or blocks the enzymatic or biological activity of the glyoxysomal malate dehydrogenase.
- 40 11. A method according to claim 8, 9 or 10, wherein
- i. either malate dehydrogenase is expressed in a transgenic organism or an organism which naturally contains malate dehydrogenase is grown,
- 45 ii. the malate dehydrogenase of step i) is brought into

contact with a test compound in the cell digest of the transgenic or nontransgenic organism, in partially purified form or in homogeneously purified form, and

5

iii. selecting a test compound which reduces or blocks the enzymatic activity of the malate dehydrogenase of step a).

10 12. A method according to claim 8, 9 or 10, which comprises the following steps:

i. generating a transgenic organism according to claim 7 or a transgenic organism comprising a nucleic acid sequence encoding a malate dehydrogenase comprising a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced by backtranslating the amino acid sequence of a functional equivalent of a SEQ ID NO:3, which has at least 63% identity with SEQ ID NO:3,

20

malate dehydrogenase being overexpressed in the transgenic organism,

25 ii. applying a test substance to the transgenic plant of i) and to a nontransgenic plant of the same genotype,

30 iii. determining the growth or the viability of the transgenic plant and the nontransgenic plant after application of the test compound, and

35 iv. selecting test substances which bring about a reduced growth of the nontransgenic plant in comparison with the growth of the transgenic plant.

13. A method according to claim 15, which is carried out in a plant organism, a cyanobacterium or proteobacterium.

40 14. A method for identifying substances with growth-regulatory activity, which comprises the following steps:

45 i. generating a transgenic plant comprising a nucleic acid sequence encoding a polypeptide with the biological activity of a glyoxysomal malate dehydrogenase comprising

- 5 a) a nucleic acid sequence according to claim 4,
or
- 10 b) a nucleic acid sequence which, owing to the
degeneracy of the genetic code, can be deduced
by backtranslating the amino acid sequence of
a functional equivalent of SEQ ID NO:3 which
has at least 63% identity with SEQ ID NO:3,
malate dehydrogenase being overexpressed in the
transgenic organism,
- 15 ii. applying a test substance to the transgenic plant
of i) and to a nontransgenic plant of the same
variety,
- 20 iii. determining the growth or the viability of the
transgenic plant and the nontransgenic plant after
application of the test compound, and
- 25 iv. selecting test substances which bring about a
reduced growth of the nontransgenic plant in
comparison with the growth of the transgenic plant.
15. A method according to any of claims 8 to 14, wherein the
substances are identified in high-throughput screening
method.
- 30 16. A support comprising one or more of the nucleic acid
molecules according to claim 2, one or more expression
cassettes according to claim 5, one or more vectors
according to claim 6, one or more organisms according to
claim 7 or one or more (poly)peptides according to claim
35 3.
- 40 17. A method according to any of claims 8 to 15, wherein the
substances are identified in High-Throughput Screening
using a support according to claim 16.
18. A compound with herbicidal activity, identified by one of
the methods according to any of claims 8 to 13, 15 and
17.
- 45 19. A compound with growth-regulatory activity, identified by
the method according to any of claims 14, 15 or 17.

20. A method for the preparation of an agrochemical composition, which comprises
- 5 a) identifying a compound with herbicidal activity by one of the methods according to any of claims 8 to 13, 15 and 17 or a compound with growth-regulatory activity according to any of claims 14, 15 or 17, and
 - 10 b) formulating this compound together with suitable auxiliaries to give crop protection products with herbicidal or growth-regulatory activity.
- 15 21. A method for controlling undesired vegetation and/or for regulating the growth of plants, which comprises allowing at least one malate dehydrogenase inhibitor according to claim 18 or 19 or an agrochemical composition comprising a malate dehydrogenase inhibitor according to claim 18 or
- 20 19 to act on plants, their environment and/or on seeds.
22. The use of a malate dehydrogenase inhibitor according to claim 18 or 19 or of an agrochemical composition comprising a malate dehydrogenase inhibitor according to
- 25 claim 18 or 19 in a method according to claim 21.
23. A method for generating nucleic acid sequences which encode malate dehydrogenase which is not inhibited by substances according to claim 18, where the nucleic acid
- 30 sequence comprises a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be derived by backtranslating the amino acid sequence of a functional equivalent of SEQ ID NO:3 which has at least 63% identity with SEQ ID NO:3;
- 35 which comprises the following process steps:
- 40 a) expression of the protein encoded by the nucleic acid sequence according to i) in a heterologous system or in a cell-free system;
 - b) randomized or directed mutagenesis of the protein by modification of the nucleic acid;
 - 45 c) measuring the interaction of the modified gene product with the herbicide;

- 5 d) identification of derivatives of the protein which show less interaction;
- 5 e) assaying the biological activity of the protein after application of the herbicide;
- 10 f) selection of the nucleic acid sequences which have modified biological activity with regard to the herbicide.
24. The method according to claim 23, wherein the sequences selected in accordance with claim 23 f) are introduced into an organism.
- 15 25. A method for the generation of transgenic plants which are resistant to substances according to claim 18, wherein a nucleic acid sequence encoding a malate dehydrogenase which comprises
- 20 a) a nucleic acid sequence according to claim 2; or
- 25 b) a functional equivalents of the nucleic acid sequence of SEQ ID NO:3 which has at least 63% identity with SEQ ID NO:3; or
- is overexpressed in these plants.
- 30 26. A transgenic plant generated by a method according to claim 25.